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THE CORRELATION BETWEEN XANTHOMONAS ORYZAE INOCULUM CONCENTRATION AND THE SIZE OF DISEASE SPOTS IN THE FLAG AND SECOND LEAVES

[Following is a translation of an article originally entitled "Effects of Inoculated Concentrations of Xanthomonas Oryzae on the Size of Disease Spots," by Shigematsu Kuhara, Environmental Section 1, in Kyushu Nogyo Shikenjo Iho (The Bulletin of the Kyushu Agricultural Experimental Station), Vol. 4, No. 1, pp 121-127.]

I. INTRODUCTION

Mukai, et al.[1,2] report that the disease spots formed following needle inoculation with Xanthomonas oryzae are small in the resistant varieties and large in the susceptible varieties. The writers[3,4] have reported the same results in similar experiments that were performed during the classification of resistant varieties. Mukai, and Yoshida,[1] have observed that there are differences in the size of the disease spots in the inoculated leaves as a function of the infection site. The spots are largest at the tips of the leaves, then diminish in size at the center and the base. The writers have performed experiments to determine the differences in the onset of the infection and the size of the disease spots as a function of leaf position and inoculum concentration following needle inoculation. As a few observations have been made on the mutual correlation between these factors, they have been summarized in this report.

Deep thanks are expressed to Mr. Konjiro Kiriu, Mr. Tadahiro Nishizawa and Mr. Michio Midome for their guidance during the conduct of these studies.

II. EXPERIMENTAL METHODS AND RESULTS

Experiment I. Experiments on the Size of the Disease Spots in the Flag and Second Leaves.

(1) During 1951, four plants each of 20 varieties were cultivated in 40 unglazed pottery, 1/10,000 tambu [1 tambu = .1 hectares] pots, the plantings being replicated. The flag and second leaves of the plants were needled inoculated with a suspension of the pathogenic fungus. It had been observed in separately conducted preparatory experiments that the disease spots which formed on the leaves tended to be larger in order from the flag leaf down through the lower leaves and that the difference between the flag and the second leaves was most marked. All of the tests made for the present report are based on comparisons of the flag and second leaves. Table I shows the size of the disease spots in the flag and second leaves of the 20 varieties tested.

The methods used for inoculation and for observation were the same as those covered in the previous report.[3]

(2) During 1952, 12 pots of the same type as those used during the previous year were utilized in the tests. These pots were planted with three varieties to replicate the tests four times. The operations were the same as those that had been conducted the prior year, while inoculation was by the same method as previously reported by the authors.[4]

The results are summarized in Figure 1.

On the basis of the data contained in Table 1 and Figure 1, it can be said that the disease spots are markedly larger in the second leaves than they are on the flag leaves in both the resistant and susceptible varieties around the heading period.

It appeared in Figure 1 that there was less difference in the area of the disease spots on the flag and the second leaves in the resistant variety, Ogyoku, than the other two varieties. For this reason, the difference in the area of the disease spots on the flag leaves and the second leaves was calculated from the data in Table 1 for each of the varieties. A rank correlation was then established between the area of the disease spots on the flag leaves and the difference between the areas of the disease spots on the flag and second leaves for all of the varieties tested. It was noted that Spearman's p for the first inspection was +0.82 and that it was +0.67 for the second inspection. This result was recognized as being significant with a 1% and 5% standard, respectively.

TABLE 1 *The Average Size of Disease Spots on Flag-Leaves and Second-Leaves by "Needle-Puncture" Inoculation.*

Varieties	First observation		Second observation	
	Flag-leaves	Second-leaves	Flag-leaves	Second-leaves
	mm^2	mm^2	mm^2	mm^2
1	6	48	81	336
2	11	47	276	581
3	2	13	125	177
4	6	23	119	213
5	35	106	283	796
6	12	40	425	923
7	6	41	233	796
8	16	82	511	1110
9	7	62	363	875
10	8	53	227	878
11	11	86	509	1272
12	44	193	1613	2399
13	12	59	225	752
14	129	459	966	1712
15	13	75	575	1706
16	17	88	230	429
17	4	33	150	371
18	6	39	267	971
19	13	53	300	517
20	11	101	530	822

Date of inoculation : Aug. 31

Date of First Observation : Sep. 15 Date of Second Observation : Oct. 1

(Total disease spot area of 24 leaves, 96 inoculated points per variety.)

Key:
1. Zensho No. 26
2. Akashinryoku
3. Shinseki No. 1
4. Ogyoku
5. Norin No. 12
6. Kogane maru
7. Seikai, No. 46
8. Kamiai
9. Oita mitsui No. 120
10. Tama
11. Nankai No. 3
12. Seikai No. 44
13. Norin No. 40
14. Toishi
15. Kamiyama
16. Norin No. 18
17. Norin No. 27
18. Norin No. 37
19. Tambo
20. Norin No. 29

Thus, it may be observed that, in the varieties in which the disease spots formed as a result of needle inoculation are small, the difference between the area of the disease spots on the flag leaves and the second leaves tends to diminish as a function of increased resistance.

Experiment 2. Experiments on Infection Rates and Inoculum Concentration

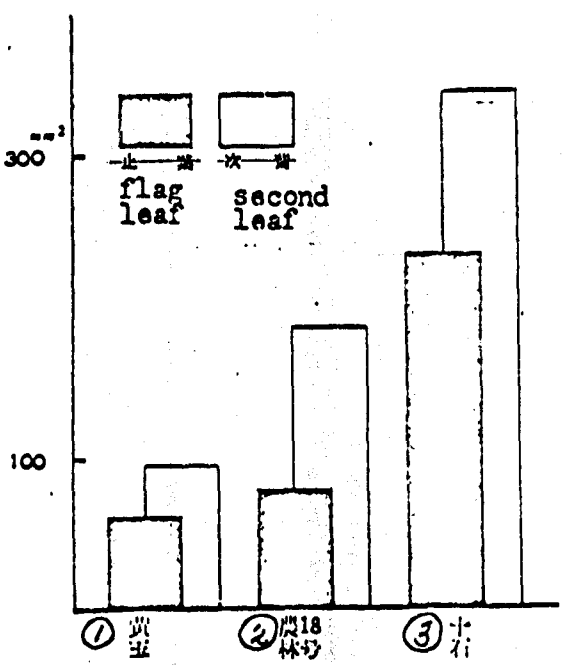
During 1952, a study was made of the correlation between inoculum concentration and infection percentages at the needle puncture sites in the wet paddy rice, Norin No. 18, in the fields. This variety is believed

to be of medium resistance. Cultivation conditions, in summary, were: Planting May 24; transplanting to the main fields, June 27; three stalks of each plant planted; space between plants, 9 sun [1 sun = 1.2 inches] X 9 sun. Fertilization (per tan [1 tan = .245 acres] was: Initial fertilization (June 25) 100 kan [1 kan = 8.72 lbs.] of compost, 6 kan of ammonium sulfate, 10 kan of lime and 3 kan of potassium chloride; first supplementary fertilization (July 18), 2 kan of ammonium sulfate; second supplementary fertilization (August 16), 2 kan of ammonium sulfate. The bacteria used for the inoculation were supplied by the Agricultural Technology Institute. The bacteria were cultured for five days at 27°C on a 2% sugar, Irish potato broth, inclined agar culture medium. From five test tubes of this culture were made 10 cc of the bacterial suspension. The suspension was diluted to a point between No. 3 and No. 4 in a standard barium sulfate solution[5] by nephelometry. This was used as the standard inoculum concentration. The test concentrations were in 13 dilutions, $1/2^0$, $1/2^1$, $1/2^2$, $1/2^3$, $1/2^4$, $1/2^5$, $1/2^6$, $1/2^7$, $1/2^8$, $1/2^9$, $1/2^{10}$, $1/2^{11}$, and $1/2^{12}$, of the standard inoculum concentration. The test groups were replicated six times. Within each test group, 13 plants were selected at random, then three stalks of each of these plants were selected at random and the code for the above concentrations attached to each of these stalks.* The centers of the flag leaves were then inoculated with six vaccine points fixed in two rows 8 mm apart at intervals of 10 mm. An inspection was made four days later of each of the leaves that had been inoculated to determine whether disease spots had formed at the inoculation puncture sites.

Fig.1. *The Average Size of Disease Spots on Flag-Leaves and Second-Leaves by "Needle-Puncture" Inoculation.*

Date of inoculation: Sep. 17 Date of observation: Oct. 2

(Total disease-spot area of 64 leaves, 256 inoculated points per variety).



Key: 1. Ogyoku
2. Norin No. 18
3. Toishi

* The same concentration was repeated for three stalks within each test group at will.

The results were as shown in Table 2.

接種菌濃度 Bacterial concentration in inoculum	反復 Replication						計 Total	接種点発病率(%) Percentage of diseased spots
	1	2	3	4	5	6		
1. 標準 × 1/2⁰	18	15	18	16	18	18	103	95.37
1/2¹	14	14	13	18	18	18	91	84.26
1/2²	17	10	13	17	8	16	81	75.00
1/2³	8	11	17	15	14	15	80	74.07
1/2⁴	6	10	14	8	8	10	56	51.85
1/2⁵	5	12	10	7	8	8	51	47.22
1/2⁶	0	5	4	8	3	5	25	23.15
1/2⁷	2	3	6	2	1	2	16	14.81
1/2⁸	1	1	2	3	0	1	7	6.48
1/2⁹	0	1	0	0	1	1	3	2.77
1/2¹⁰	0	0	1	1	0	0	2	1.85
1/2¹¹	0	0	1	0	0	0	1	0.93
1/2¹²	0	0	1	0	0	0	1	0.93

接種月日：9月18日 Date of inoculation : Sep. 18　　調査月日：10月17日 Date of observation : Oct. 17　　(1区あたり18接種点)

Key: 1. Standard
2. (18 inoculation points per group)

As is evident in Table 2, there is a reduction in the infection rate at the inoculation points as a function of a reduction in the bacterial concentration of the inoculum.

Figure 2 shows the correlation between the percentage of disease spots at the points of inoculation and the bacterial concentration in the inoculum as drawn on a normal probability graph.

As the correlation between both of these factors as seen in the figure is generally linear,* it is believed that the correlation between the logarithm of the inoculum concentration and the percentage of disease spots at the point of inoculation will generally follow a normal sigmoid curve.

Experiment 3. Tests of the Infection Rate and the Size of the Spots on the Flag and Second Leaves and Inoculum Concentration

During 1952, a study was made of the effects of a reduction in the bacterial concentration of the inoculum on infection rates and the size of the disease spots on the flag leaves and the second leaves. The bacterial concentration of the inoculum was reduced in ten groups comprising $1/3^0$, $1/3^1$, $1/3^2$, $1/3^3$, $1/3^4$, $1/3^5$, $1/3^6$, $1/3^7$, $1/3^8$, and $1/3^9$ dilutions of the standard inoculum concentration. A group of 10 plants was replicated

* The results of linearity tests revealed the highly significant fact that the variance in the replication was 185.69**.

(total of 20 plants) and three stalks were taken at random from each plant. Treatment code numbers were attached at random to these stalks. The centers of the flag and second leaves were then inoculated with 10 vaccine points fixed in two rows 8 mm apart at intervals of 10 mm. The varieties tested and the bacterial inoculum were the same as those used in Experiment 2. Cultivation was in accordance with the cultivation standards for wet paddy rice as specified by the Kyushu Agricultural Experimental Station. An inspection was made four days subsequent to the inoculation to determine the number of inoculation points infected. Measurements were also made of the size of the disease spots at the infected inoculation points.

The results obtained were as shown in Table 3 and Figure 3.

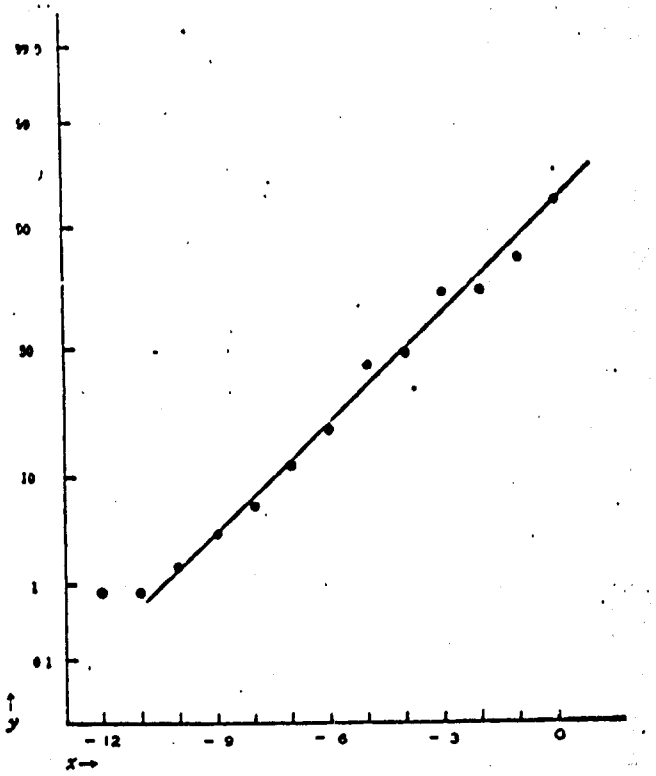


FIG.2. *The Percentage of Disease Spot Plotted on the Probability Paper Corresponding to Logarithms of Bacterial Concentration in Inoculum.*
$x = \log_2 A$
y : Percentage of disease spot.
A : Bacterial concentration in inoculum.

TABLE 3 *Difference between Flag-Leaves and Second-Leaves in the Percentage of Diseased Spot in Relation to the Inoculum Concentration.*

接種菌濃度 Bacterial concentration in inoculum	葉位 Leaf order	
	止葉 Flag-leaves	次葉 Second-leaves
	%	%
基準 × 1/3⁰	100.00	100.00
1/3¹	100.00	100.00
1/3²	96.66	100.00
1/3³	48.33	96.66
1/3⁴	28.33	70.00
1/3⁵	16.66	23.33
1/3⁶	8.33	18.33
1/3⁷	5.00	16.66
1/3⁸	5.00	25.00
1/3⁹	1.66	15.00

接種月日：9月18日 Date of inoculation : Sep. 18　調査月日：10月17日 Date of observation : Oct. 17　(1区60接種点) (2)

Key: 1. Standard
2. (60 inoculation points per group)

The values shown in Table 3 were derived as follows: (Total disease spot area)/(number of infected inoculation sites) was calculated for each leaf. The results were then totaled for all of the leaves which had infected inoculation sites. That product was divided by the total number of leaves to give the average disease spot area for each inoculation point for all of the diseased leaves in each inoculum concentration group.

It may be observed from Table 3 and Figure 3 that the size of the disease spots drops as a function of reductions in the concentration of the inoculum. The disease spots generally form at lower concentrations in the second leaves, which have lower resistance, than in the flag leaves and the area of these spots is greater.

III. DISCUSSION

It has been generally recognized that there are differences in susceptibility to disease damage as a function of leaf order and the age of the leaves[6,7,8,9,10]. Toyama,[6] has reported that absolutely no wheat snow rot disease lesions form on the young leaves, but that the percentage of disease lesions increases as a function of increased leaf age. He concludes that there are substantial differences in infection between the different leaf positions.

With regard to the differences in the resistance among plant varieties and differences in infection as a function of leaf order, Simon[7] states that there are virtually no differences in the formation of oat crown rust uredia in the resistant varieties as a function of leaf

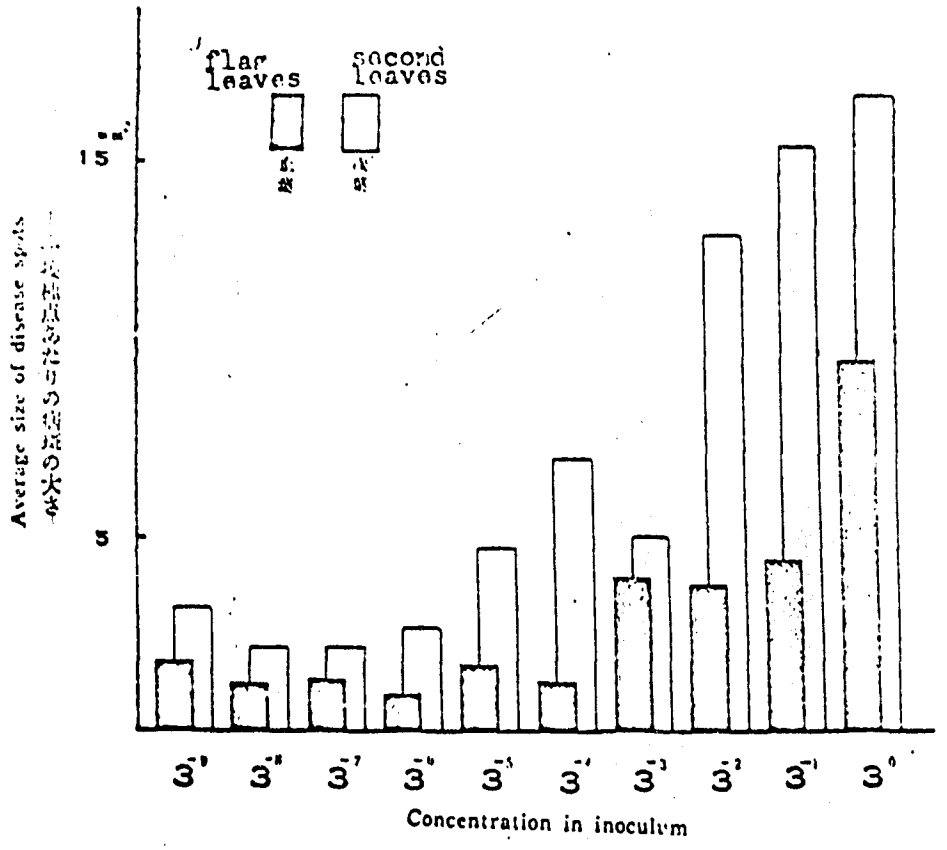


Fig.3. *Relation between the Size of Disease spot and the Bacterial Concentration in Inoculum.*

age, but that there are greater differences among the varieties in which resistance varies by development stage and temperature. He also states that the uredia form in gradually increasing numbers in the susceptible varieties from the top leaves down, but that there are virtually no differences between the leaves in the resistant varieties. It is believed that the same sort of correlation may be observed in Xanthomonas oryzae in the present experiments. It is therefore considered that the generally heavier infection by this disease in the lower leaves in the fields, in addition to being attributable to the timing of the infection and other outbreak conditions, may be also attributed to differences in resistance as a function of leaf position.

As a general rule, there is a close correlation in animals, between death rates or infection rates and the bacterial or viral concentration of the inoculum (or the quantity of the inoculation). It is well known that this correlation forms a sigmoid curve. There has been wide use of probit and LD_{50} in studies comparing the pathogenicity of bacteria and for estimating viral concentration[5,11,12]. The writers, have not seen any reports of the application of this methodology to plant pathogenic bacteria however. Price, et al. [13,14,15] have reported on plant virus diseases and

discuss the reduction in the number of necrotic lesions as a function of reductions in inoculum concentration in a number of virus diseases. They have demonstrated that the log-ratio method can be used for the measurement of viral concentration. As noted in Experiments 2 and 3, there is a reduction in infection rate as a function of reductions in the bacterial concentration in the inoculum. By applying an appropriate methodology, it is therefore believed that it is possible to estimate to a degree the quantity of bacteria and their pathogenicity as well as resistance from this correlation. For example, it is probably possible to use probit analysis for determining pathogenicity, resistance and the quantity of bacteria when the curve of the correlation between bacterial concentration in the inoculum and infection rate is sigmoid as in Experiment 2.*

With regard to the sigmoid correlation between inoculum concentration and infection rates, however, it is believed that more careful tests must be made when there are differences between the resistance of the rice plants, pathogenicity, bacterial concentration or other conditions.**

Comparing the results of Experiment 3 to those of Experiment 2, the infection rate for given bacterial concentrations was higher in the former although the bacteria used for the inoculations and the rice plant varieties were the same. Just as Spencer, et al.[15] have cautioned with regard to the formation of necrotic lesions in tobacco mosaic, this difference may be accepted as demonstrating that the differences in cultivation methods and environmental conditions will affect the correlation between bacterial concentration and disease rates even in the same plant variety.

IV. SUMMARY

A study was made of the differences in Xanthomonas oryzae infection rates and the size of the disease spots as functions of leaf order and bacterial concentration by the needle inoculation of the flag leaves and second leaves. The results may be summarized as follows.

1. The disease spots which form at the inoculation sites on the second leaves are much larger than those on the flag leaves when the two are inoculated concurrently.

2. Among those varieties in which the disease lesions in the flag leaves are small, i.e., in the resistant varieties in which the difference in the areas of the disease lesions on the flag leaves and the second leaves is small, the difference tends to increase in the varieties in which area of the disease lesions in the flag leaves was larger.

*When the probit method is used, the infection rate in Experiment 2 is still similarly linear.

**The results of the tests of linearity on the probit transformation of infection rates as derived from Experiment 3 are significant, but their applicability is no better than in Test 2.

3. The rate at which disease lesions appears in the infected inoculation sites decreases as a function of decreases in the bacterial concentration of the inoculum. The size of the lesions also decreases.

4. The curve of the correlation between the logarithm of inoculum concentration and the infection rate at the inoculation site is a normal sigmoid curve, however, the applicability of the sigmoid curve to the results of Experiment 3 was less than its applicability to the results of Experiment 2.

5. Compared to the flag leaf, the less resistant second leaf is more easily infected by lower bacterial concentrations and the disease lesions are larger.

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SUMMARY

Percentages and size of diseased spots on the flag- and the secondary-leaves lineally decreased with gradually decreasing inoculated bacterial concentrations. The diseased spots of the secondary-leaves were remarkably larger in size than those of the flag-leaves, which were found to be more resistant than the secondary-leaves regardless of bacterial concentrations. In the secondary-leaves, diseased spots could easily be formed at very low bacterial concentrations. The differences in spotted area between the flag- and the secondary-leaves were smaller in resistant varieties than in the susceptible varieties.